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Chromosomal location of heterochromatin and 45S rDNA sites in four South American triatomines (Heteroptera: Reduviidae)

V.B. Bardella[1], M.L. Gaeta[2], A.L.L. Vanzela[2], M.T.V. Azeredo-Oliveira[1]

[1]Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (UNESP/IBILCE), São José do Rio Preto, São Paulo, Brazil. [2]Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil.

E-mails: [1]tercilia@ibilce.unesp.br, [2]andrevanzela2008@gmail.com

Abstract. The Triatominae are distributed in Brazilian regions and are the vector of Chagas disease. This group is also characterized by lower karyotype variability, occurrence of holokinetic chromosomes and inverted meiosis of the sex chromosomes. In this study, we determined the karyotype of four species of the genus *Triatoma* Laporte, 1832 using chromosome measurement, chromosome banding and FISH with a 45S rDNA probe. All samples showed 2n = 20A+XY with chromosomes sorted by size in decreasing order. Heterochromatin of *Triatoma infestans melanosoma* Lent, Jurberg, Galvão, Carcavallo, 1994 is distributed among eight autosomes and the sex chromosomes. In *Triatoma brasiliensis* Neiva, 1911, *T. matogrossensis* Leite, Barbosa, 1953 and *T. rubrovaria* Blanchard, 1834, heterochromatin was restricted to the Y chromosome, which was characterized as DAPI$^+$ (weak signal). FISH using a 45S rDNA probe of *Drosophila melanogaster* Meigen, 1830 showed differences in the number and location of hybridization sites. *T. brasiliensis* and *T. rubrovaria* showed the signal on one autosome pair. *T. matogrossensis* showed signals on both sex chromosomes, and *T. infestans melanosoma* only on the X chromosome. Conventional banding analysis suggests a closer relationship between *T. brasiliensis*, *T. matogrossensis* and *T. rubrovaria*, except with regard to 45S rDNA location in *T. matogrossensis,* and a more differentiated karyotype in *T. infestans melanosoma*.

Key words: FISH, holokinetic chromosomes, repetitive DNA, *Triatoma*, 45S rDNA.

Introduction

The Triatominae are an important insect group due to their association with Chagas disease, which affects about 13 to 15 million people in the Americas (Mendonça et al., 2009). In addition to their epidemiologic importance, triatomines are also interesting because their chromosomes do not show morphological differentiation originating from the centromeric region (Hughes-Schrader, Schrader, 1961). Insects belonging to the suborder Heteroptera, like the orders Hemiptera, Lepidoptera and Trichoptera, possess holocentric (=holokinetic) chromosomes (Wolf, 1996). During mitosis the holokinetic chromatids move in a parallel manner due to a diffuse kinetochore, and this diffuse condition permits the occurrence of intra- and interspecific karyotype variations

in insects due to agmatoploidy and symploidy events. In meiosis there is some evidence of post-reductional condition for the sex chromosomes (Solari, 1979; Ueshima, 1979). Additionally, like majority of heteropteran species (Ueshima, 1979), the autosomes in *Triatoma* species are chiasmate and the sex chromosomes are achiasmate.

The common karyotype in the Triatominae is 2n = 20A + XY (Panzera et al., 1996), and banding studies have shown few heterochromatic bands, located frequently in the terminal regions of chromosomes (Pérez et al., 1997). The use of CMA_3/DAPI banding revealed, as in most insects, GC-rich blocks associated with rDNA sites (Mandrioli et al., 1999; Rebagliati et al., 2003; Golub et al., 2004). Studies involving rDNA localization in heteropterans have shown sites on autosomes and sex chromosomes (González-García et al., 1996; Cabrero et al., 2003; Cattani, Papeschi, 2004).

The cytogenetics of South American triatomines is not well explored and the lack of information about chromosome markers and physical mapping has prevented the establishment of karyotype relationships. Previous chromosome studies in South American species reported mainly aspects of chromosome number and shape of *Triatoma infestans melanosoma* Lent, Jurberg, Galvão, Carcavallo, 1994 (=*T. melanosoma*; Galvão et al., 2003) (Monteiro et al 1999; Bardella et al., 2008), *T. brasiliensis* Neiva, 1911, *T. matogrossensis* Leite, Barbosa, 1953 and *T. rubrovaria* Blanchard, 1834 (De Vaio et al., 1985; Panzera et al., 2010). Thus, the aim of this study was to detect heterochromatic segments and rDNA sites in order to elucidate a possible distribution pattern of these segments and to establish the karyotype organization and relationships in these four South American triatomines.

Material and methods

Chromosome preparation

Adult males of *Triatoma brasiliensis* (15 specimens), *T. infestans melanosoma* (14), *T. matogrossensis* (11) and *T. rubrovaria* (10) were obtained from the insectary of the Faculdade de Ciências Farmacêuticas, Departamento de Ciências Biológicas UNESP (Araraquara-SP) and from Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos – LNIRTT FIOCRUZ (Rio de Janeiro- RJ), both in Brazil.

The gonads were dissected out and the seminiferous tubules were fixed in methanol-acetic acid (3:1, v:v) and then stored at -20 ºC. For slides, the tubules were washed twice in distilled water for 5 minutes (to remove the fixative), and they were incubated in 45% acetic acid, and then dilacerated and squashed. The coverslips were removed after freezing in liquid nitrogen.

Chromosomes were measured using two meiotic metaphases for each of five different individuals of each species. The measurement was performed manually, using a needle point compass. Chromosome pairs were arranged in decreasing size, according to average size and standard deviation.

Chromosome banding

C-banding was performed as proposed by Sumner (1982) with modifications. Slides were incubated in 0.2 N HCl for 10 min. at room temperature, 5% barium hydroxide for 7-15 min. at 60 ºC and 2× SSC, pH 7.0, for 60 min. at 60 ºC. The slides were dehydrated in 70% and 100% ethanol for 10 min. each (to remove the water from the material). The samples were air-dried and stained with fluorochromes: 0.5 mg/mL CMA_3 for 1.5 h and 2 µg/mL DAPI for 30 min. Slides were mounted with a medium composed of glycerol/McIlvaine buffer, pH

Table 1. Chromosome measurements (mean and standard deviation) of four species of *Triatoma* from South America. Y and X – sex chromosomes.

Pair	*T. infestans*	*T. brasiliensis*	*T. matogrossensis*	*T. rubrovaria*
1	16.68 ±1.15	12.40 ±1.10	12.16 ±0.96	11.81 ±0.69
2	13.88 ±1.36	11.11 ±0.69	10.92 ±0.65	11.04 ±0.69
3	12.24 ±1.47	10.27 ±0.68	10.16 ±0.60	10.48 ±0.70
4	8.57 ±0.56	9.70 ±0.49	9.68 ±0.51	9.75 ±0.32
5	7.66 ±0.41	9.16 ±0.25	9.05 ±0.49	9.19 ±0.41
6	6.98 ±0.35	8.76 ±0.33	8.62 ±0.51	8.71 ±0.36
7	6.59 ±0.60	8.50 ±0.26	8.37 ±0.42	8.24 ±0.40
8	6.36 ±0.67	8.19 ±0.18	8.02 ±0.26	8.15 ±0.30
9	6.11 ±0.62	7.74 ±0.53	7.59 ±0.63	7.38 ±0,51
10	5.40 ±0.56	7.08 ±0.49	6.97 ±0.54	6.68 ±0.53
Y	5.52 ±0.57	4.24 ±0.42	4,81 ±0.49	5.16 ±0.49
X	3.73 ±0.47	3.15 ±0.34	3.92 ±0.42	3.72 ±0.45

7.0 (1:1, v:v), plus 2.5 mM $MgCl_2$.

Fluorescence in situ hybridization

The fluorescent *in situ* hybridization (FISH) was performed as described in Vanzela et al. (2002) for plant chromosomes. Slides were prepared as described for conventional staining and immediately used for FISH. The fragment p*Dm*238, containing the 18S, 5.8S and 28S genes of *Drosophila melanogaster* Meigen, 1830 (Roiha et al., 1981), was labeled with biotin-14-dATP by nick translation. Preparations were incubated in 100 µg/mL RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in 70-100% ethanol series and air-dried. Slides were treated with 30 µL of hybridization mixture containing 100 ng of labeled probe (4 µL of probe), 15 µL of 100% formamide (50%), 6 µL of 50% polyethylene glycol (10%), 3 µL of 20× SSC (2×SSC), 1 µL of calf thymus DNA (100 ng) and 1 µL of 10% SDS (0,33%). Chromosome denaturation/renaturation was done at 90 °C for 10 min, 50 °C for 10 min and 38 °C for 10 min using a thermal cycler (MJ Research), and hybridization was performed overnight at 37 °C in a humidified chamber. Post-hybridization washes were carried out in 2× SSC, 20% formamide in 0.1× SSC, 0.1× SSC and 4× SSC/0.2% Tween 20, all at 37 °C. The probe was detected with a solution composed of 5 µg/mL avidin-FITC (fluorescein isothiocyanate) and 5% BSA/4× SSC/0.2% Tween 20 (1:100, v:v). The post-detection washes were performed in 4× SSC/0.2% Tween 20 at room temperature. Slides were mounted with 26 µL of a medium composed of 23 µL of DABCO solution (1,4-diaza- bicyclo (2.2.2)-octane (2,3%), 20 mM Tris HCl, pH 8.0, (2%) and glycerol (90%), in distilled water), 2 µL of 2 µg/mL DAPI and 1 µL of 50 mM $MgCl_2$.

Image capture

All images were acquired in a grayscale mode using a Leica DM 4500 B microscope, equipped with a DFC 300FX camera. For FISH the images were pseudocoloured (red for DAPI and greenish-yellow for FITC) and overlapped using the Leica IM50 4.0 software. The images were optimized for best contrast and brightness with iGrafx Image software.

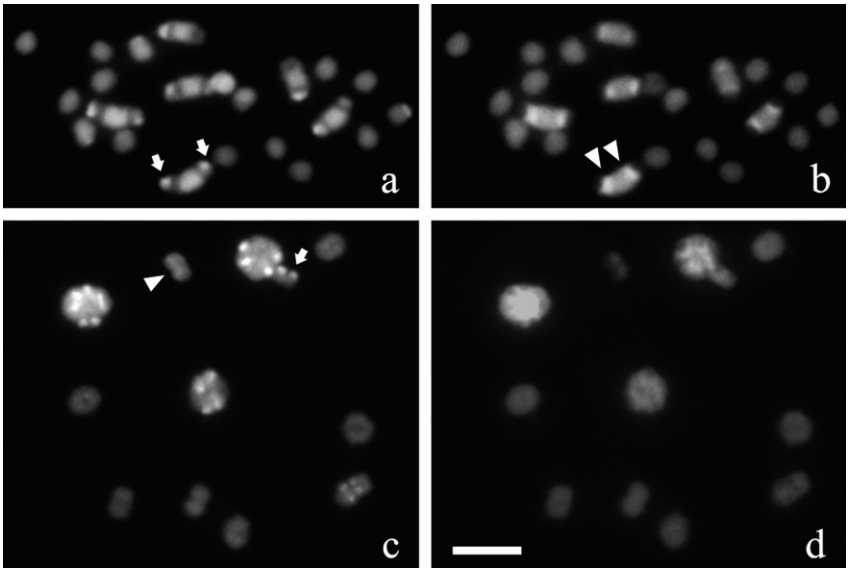

Fig. 1. a-d. Metaphase cells of *Triatoma infestans melanosoma* submitted to C- DAPI/ CMA_3 staining. **a, b** - Mitotic metaphase showing three autosome pairs with terminal blocks, $DAPI^+$ (**a** - arrow) and adjacent to GC-rich blocks (**b** - arrowhead). **c, d** - Metaphase I with four autosomes showing terminal $DAPI^+$ blocks (**c**) and three autosomes with CMA_3^+ blocks (**d**). Also note the Y chromosome completely $DAPI^+$ (**c** - a weak signal, arrowhead) and the X chromosome with a terminal $DAPI^+$ block (**c** - arrow). Bar = 5 μm.

Results

All species showed a karyotype of 2n = 20A+XY. The chromosomes were sorted by size in decreasing order (Table 1). This feature was accentuated in *T. infestans melanosoma,* since the autosomes can be organized into two groups (Fig. 1 and Table 1). The group A comprised three large pairs (1, 2 and 3), representing 42.8% of the karyotype size whereas the group B, with seven pairs (4, 5, 6, 7, 8, 9 and 10), made up 47.67% of the karyotype size (Fig. 1 and Table 1). The Y chromosome has a median size and was larger than the X chromosome (Table 1). *T. infestans melanosoma* showed heterochromatin in both terminal regions of the three large autosomes pairs (group A). In these cases, terminal AT-rich blocks with adjacent GC-rich blocks were visualized (Fig. 1, a-b). AT-rich blocks were also found in one autosome bivalent of the group B (Fig. 1, c-d). Both sex chromosomes of *T. infestans melanosoma* exhibited AT-rich heterochromatin: the Y chromosome was totally bright (weak signal), whereas the X showed only one block at one chromosome end (Fig. 1, c). FISH with the 45S rDNA probe showed a hybridization signal on the X chromosome, adjacent to an AT-rich region (Fig. 3, a).

T. brasiliensis, *T. matogrossensis* and *T. rubrovaria* showed similar karyotypes with chromosome size decreasing in the same manner (Table 1). The sex chromosomes were smaller than the autosomes, with the Y larger than the X (Fig. 2). In the three above mentioned species, the heterochromatin extended over all Y chromosome and was $DAPI^+$ (Fig. 2, a, c, e) and CMA^- (Fig. 2, b, d), except in *T. matogrossensis* which showed a CMA^+ dot (Fig. 2, e-f). FISH with the 45S rDNA probe showed a signal on one autosome pair, except

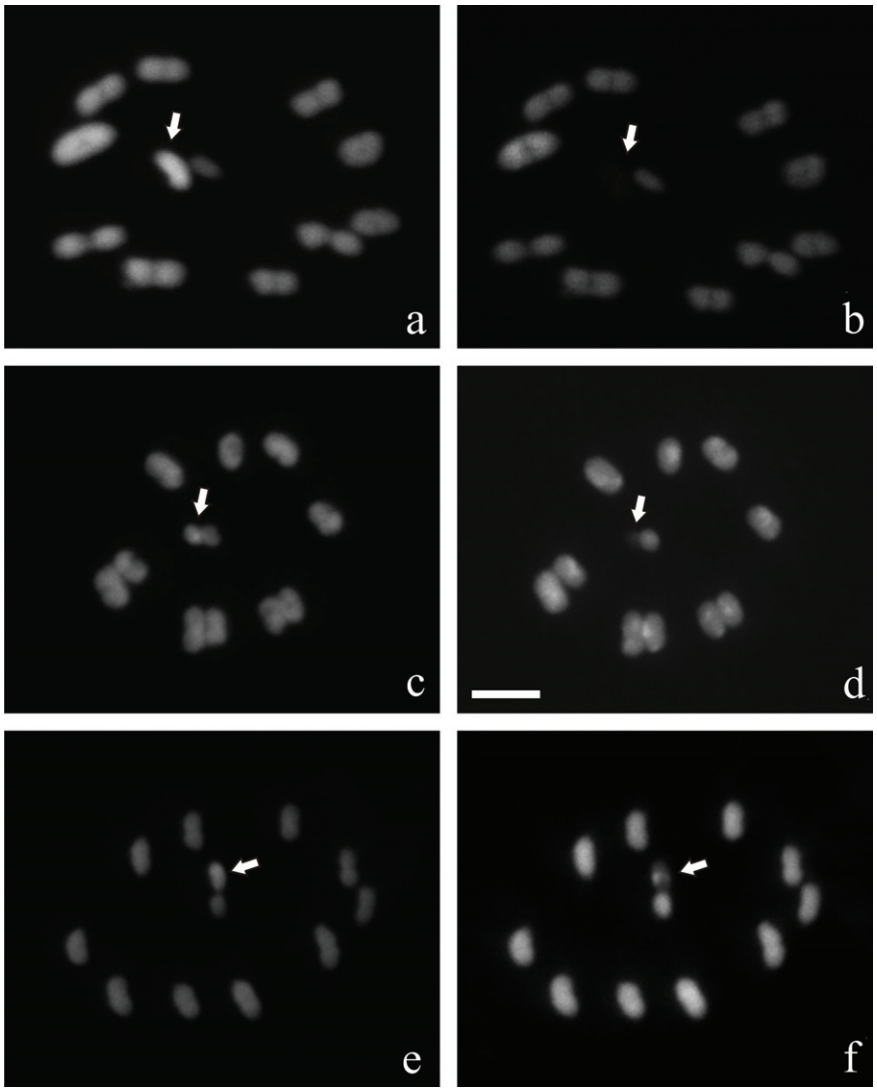

Fig. 2, a-f. Cells of *Triatoma rubrovaria* (**a, b**), *T. brasiliensis* (**c, d**) and *T. matogrossensis* (**e, f**) submitted to C- DAPI/CMA_3 staining. **a, b -** Metaphase I of *T. rubrovaria* showing the Y chromosome $DAPI^+$ (weak signal) (**a** - arrow). **c, d** – *T. brasiliensis* with Y totally $DAPI^+$ (**c** - arrow). **e, f** – *T. matogrossensis* showing Y chromosome with $DAPI^+$ and CMA^+ block (arrow). Bar = 5 μm.

for *T. matogrossensis*, where the hybridization signals were located on the sex chromosomes (Fig. 3, b-d). The intensity of the signal was different among all species (Fig. 3), however, *T. rubrovaria* and *T. matogrossensis* showed remarked differences in signal intensity between chromosomes of a pair, precisely in one bivalent of autosomes in *T. rubrovaria* (Fig. 3, d) and in the sexual chromosomes of *T. matogrossensis* (Fig. 3, b, d).

Discussion

The chromosome complement of 2n = 20A+XY is very common in the Triatominae (Ueshima, 1979; Panzera et al., 1996). In few heteropterans, the bivalents exhibit a gradual

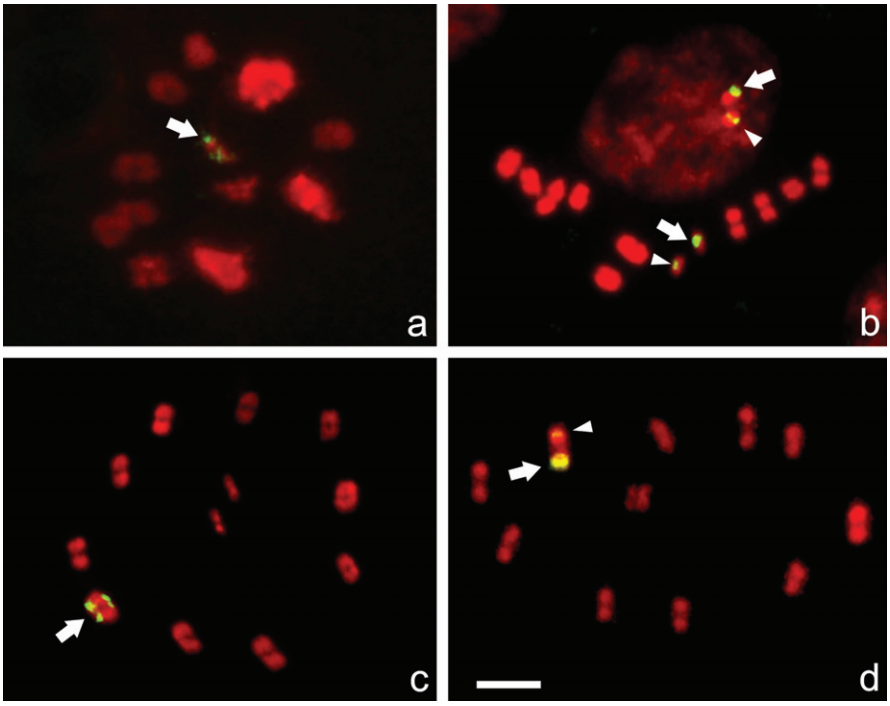

Fig. 3, a-d. Location of rDNA sites by FISH at metaphase I of *T. infestans melanosoma* with rDNA site on the X chromosome (**a -** arrow) and at metaphase I of *T. matogrossensis* with rDNA site on the sex chromosomes (**b -** arrow). **c -** *T. brasiliensis* and **d -** *T. rubrovaria.* Signals on a pair of autosomes are arrowed. Note the difference in the intensity of hybridization signals between sex chromosomes of *T. matogrossensis* (**b -**arrow) and between autosomes of a pair of *T. rubrovaria* (**d** - arrowhead). Bar = 5 µm.

decrease in size, like observed in some species of Aradidae (Jacobs, 2003), Nabidae (Kuznetsova et al., 2004) and Coreidae (Bressa et al., 2008). Jacobs (2003) suggested that the decrease in size could be a factor for grouping species, and also supposed that this feature possibly appears in ancestral species, as occurs in the aradid genus *Dundocoris* Hoberlandt, 1952 (Jacobs, 2003). The similarity in chromosome size suggests a close relationship between *T. brasiliensis*, *T. matogrossensis* and *T. rubrovaria*, as proposed in phylogenetic studies (Schofield, Galvão, 2009), when compared to *T. infestans melanosoma.* The karyotype of this last species possesses similarities with that of *T. infestans* described by Pérez et al. (1997 and 2000) and with *Pachylis argentinus* Berg, 1879 (Coreidae) reported by Papeschi et al. (2003). In all cases, the chromosome pairs were grouped into larger and smaller ones. The occurrence of a Y chromosome always larger than the X in these four species is in accordance with studies of the *Triatoma* by Pérez et al. (1992). These results reinforce the karyotype differentiation between hematophagous species, frequently with the Y larger than the X, and phytophagous species, often with the X chromosome larger than the Y (Rebagliati et al., 2003; Cattani et al., 2004).

Chromosome banding showed heterochromatin restricted to the Y chromosome of *T. brasiliensis*, *T. matogrossensis* and *T. rubrovaria*, in contrast to other heteropterans,

which exhibit heterochromatin distributed on both autosomes and sex chromosomes (Lanzone, De Souza, 2006), as also found in *T. infestans melanosoma*. The terminal location of heterochromatin seems to be a common characteristic in Heteroptera, but interstitial blocks were also reported (Camacho et al., 1985; Groveza et al., 2004; Rebagliati et al., 2003). A new feature found here was the occurrence of adjacent AT-rich blocks to GC-rich blocks, both of them located on the first three pairs of *T. infestans melanosoma*. Commonly, most of the studies reported that the heterochromatic blocks are either $DAPI^+$ or CMA^+ exclusively or with both (Rebagliati et al., 2003). In general, terminal CG-rich blocks are associated with rDNA sites in Heteroptera, like occurred in *Spartocera fusca* (Thunberg, 1783) (Coreidae) and in *Camptischium clavipes* (Fabricius, 1803) (Coreidae) (Cattani, Papeschi, 2004; Cattani et al., 2004). *T. matogrossensis* also showed this pattern; however, in *T. brasiliensis* and *T. rubrovaria*, 45S rDNA sites were not associated with GC-rich blocks, and in *T. infestans melanosoma*, the association was with AT-rich regions. Fossey and Liebenberg (1995) were the first to report an association of 45S rDNA sites with AT-rich regions in *Carlisis wahlbergi* Stål, 1858 (Coreidae: Heteroptera).

Studies involving the physical location of rDNA in heteropterans are scarce and showed that these segments can occur either only on the sex chromosomes, like in *Graphosoma italicum* (O.F. Müller, 1766) (Pentatomidae) and in *Triatoma platensi*s Neiva, 1913 (Reduviidae), or in one autosome pair, like in *T. tibiamaculata* (Pinto, 1926) (Reduviidae) and in *Spatocera fusca* (Coreidae) (Gonzáles-Garcia et al., 1996; Cattani, Papeschi, 2004; Campos Severi-Aguiar, Azeredo-Oliveira, 2005; Bressa et al., 2008). Likewise, the four species studied here exhibited this tendency; *T. matogrossensis* and *T. infestans melanosoma,* showed the 45S rDNA on the sex chromosomes, but *T. brasiliensis* and *T. rubroviaria* have one on the autosomes. Differences were also detected in the signal intensity among all species and within the karyotype (*T. matogrossensis* and *T. rubrovaria*). This situation probably reflects variation in the number of copies of the rDNA, like occurred in *Holhymenia rubiginosa* Breddin, 1904 (Coreidae) (Bressa et al., 2008). In the case of variation within the karyotype studied in this paper, we suggest that this is a consequence of unequal crossing-over events or chromosome rearrangements, i.e. duplication. However, since the sex chromosomes in triatomines are achiasmate (Solari, 1979), the first hypothesis would be rejected for *T. matogrossensis*, improving the chance of the other mechanisms operating in this species.

This cytogenetic study enabled us to show a closer karyotype relationship between *T. brasiliensis*, *T. rubrovaria* and *T. matogrossensis*, and the distant association with *T. infestans melanosoma*. However, other studies with larger numbers of species would be necessary to determine the evolutionary ways of karyotype differentiation and the plesiomorphic and apomorphic conditions in the Triatominae.

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